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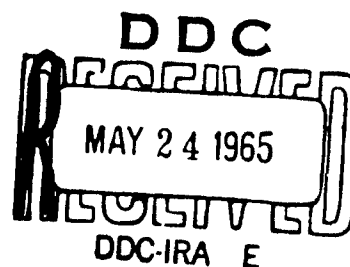
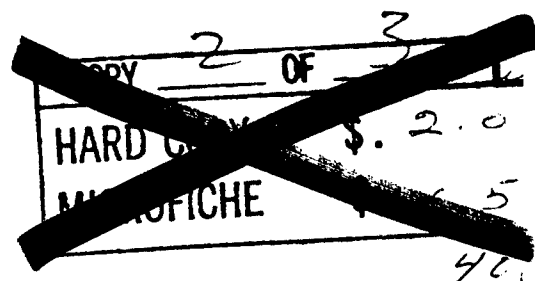
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ANNUAL REPORT ON
THERMAL AND ELECTRICAL CONDUCTIVITIES
OF BIOLOGICAL FLUIDS AND TISSUES
ONR CONTRACT NO. 4095 (00)

Period

April 1, 1964 to March 31, 1965

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SUMMARY

This report describes Geoscience's research on thermal and electrical conductivities of biological fluids and tissues for the Medicine & Dentistry Branch of the Office of Naval Research (Contract No. 4095(00), A-1) for the period of April 1, 1964 to March 31, 1965.

Experimental electrical conductivities of human gastric juices, urine and bovine aqueous and vitreous humours were determined. Special cells for the determination of electrical conductivities for tissues has been designed, constructed, and tested. Experimental measurements of the electrical conductivity of bovine blood were determined as a function of hemolysis. Changes in the electrical conductivity of hemolyzed blood have been related to erythrocyte damage.

A modified thermal conductivity apparatus was used to measure the conductivities of bovine lung, liver, kidney, brain, bone marrow, and aqueous and vitreous humour, and chicken skin. The correlations between thermal conductivity and physical properties of biological tissues and fluids were studied.

Investigations of the effect of freezing bovine liver in liquid nitrogen on thermal conductivity have been conducted. The techniques of freezing blood in liquid nitrogen have been examined also. Cryogenically frozen and stored blood samples from the Presbyterian Medical Center in San Francisco were thawed and electrical and thermal conductivity measurements were made and the results analyzed.

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I. INTRODUCTION

This research project consists of the determination of the thermal and electrical conductivities of biological fluids and tissues that have been exposed to abnormal physical fields such as temperature and radiation; particular emphasis is given to cryogenic temperature exposure. It is believed that morphological and biochemical alterations in biological fluids and tissues can be detected by measuring resulting changes in thermal and particularly electrical properties. The objective of the program is to establish the relations between changes in the physical properties of biological specimens and the physical stresses imposed upon them.

The thermal conductivities of fluids and tissues are measured using specially constructed apparatus. The electrical conductivities of fluids are determined with commercial equipment; the electrical conductivities of tissues are measured utilizing a specially constructed apparatus. The biological materials being studied are: blood, plasma, cerebrospinal fluid, bone marrow, urine, gastric juice, brain, liver, skin, heart, lung, kidney, stomach, muscle blocks, fatty tissue and tumor tissue. The biological specimens are obtained from animals and humans. Supporting measurements are made to insure the proper biological condition of the specimens; microscopic examinations and biochemical tests are performed and water and lipid contents determined.

The following sections of the report summarize 1) electrical conductivity studies, 2) thermal conductivity studies, 3) cryogenic experiments, and 4) studies to be conducted during the next year.

II. ELECTRICAL CONDUCTIVITIES

A. Blood

Present efforts in blood plasma conductivity research are being directed toward the establishment of a correlation between plasma conductivity and hemolysis (gram % hemoglobin). As described in the previous report⁽¹⁾, the total gram percentage of hemoglobin in the plasma is measured by the cyanmethemoglobin technique. A lengthy series of conductivity determinations of bovine blood plasma containing varying amounts of hemoglobin was made between 26° C and 46° C. These data are shown in Figure 1 plotted versus temperature with gram percentage hemoglobin content as the parameter. A more useful presentation of the data for 28° C may be seen in Figure 2 where the dimensionless conductivity ratio K/K_0 is plotted against hemolysis values. The value K_0 denotes the basic conductivity of the plasma with no hemoglobin present (before damage). Knowing electrical conductivity values, this general curve can then be used to ascertain the blood hemoglobin content.

Initially, a sample of normal, fresh heparinized blood was centrifuged to obtain a quantity of plasma, and another portion of the same sample was frozen and thawed to hemolyze the cells and free the hemoglobin. Various percentage solutions of the plasma and the hemoglobin were then prepared. Subsequently, the determination of the hemoglobin concentration of the plasma hemoglobin mixtures, and the measurement of their electrical conductivities was carried out.

If the mixture contains 1 gr Hb/100 ml of solution or more, its hemoglobin concentration was measured by the cyanmethemoglobin technique, in which a reagent containing $K_3Fe(CN)_6$ and KCN completely lyses the normal erythrocytes and converts all forms of hemoglobin except sulfhemoglobin to cyanmethemoglobin. The resulting stable color complex is related to the amount of hemoglobin present. From a colorimeter calibration curve of the optic density of cyanmethemoglobin versus the equivalent gr Hb/100 ml, one can determine to within $\pm 3\%$ the hemoglobin concentration of any sample containing 1 gr/100 ml or more. If a sample contains less than this amount, its concentration is measured by the orthotolidine technique. The latter method is based on the fact that Hb has a peroxidase property and will catalytically decompose H_2O_2 with the liberation of O_2 . The free oxygen results in the oxidation of the dye orthotolidine to a blue

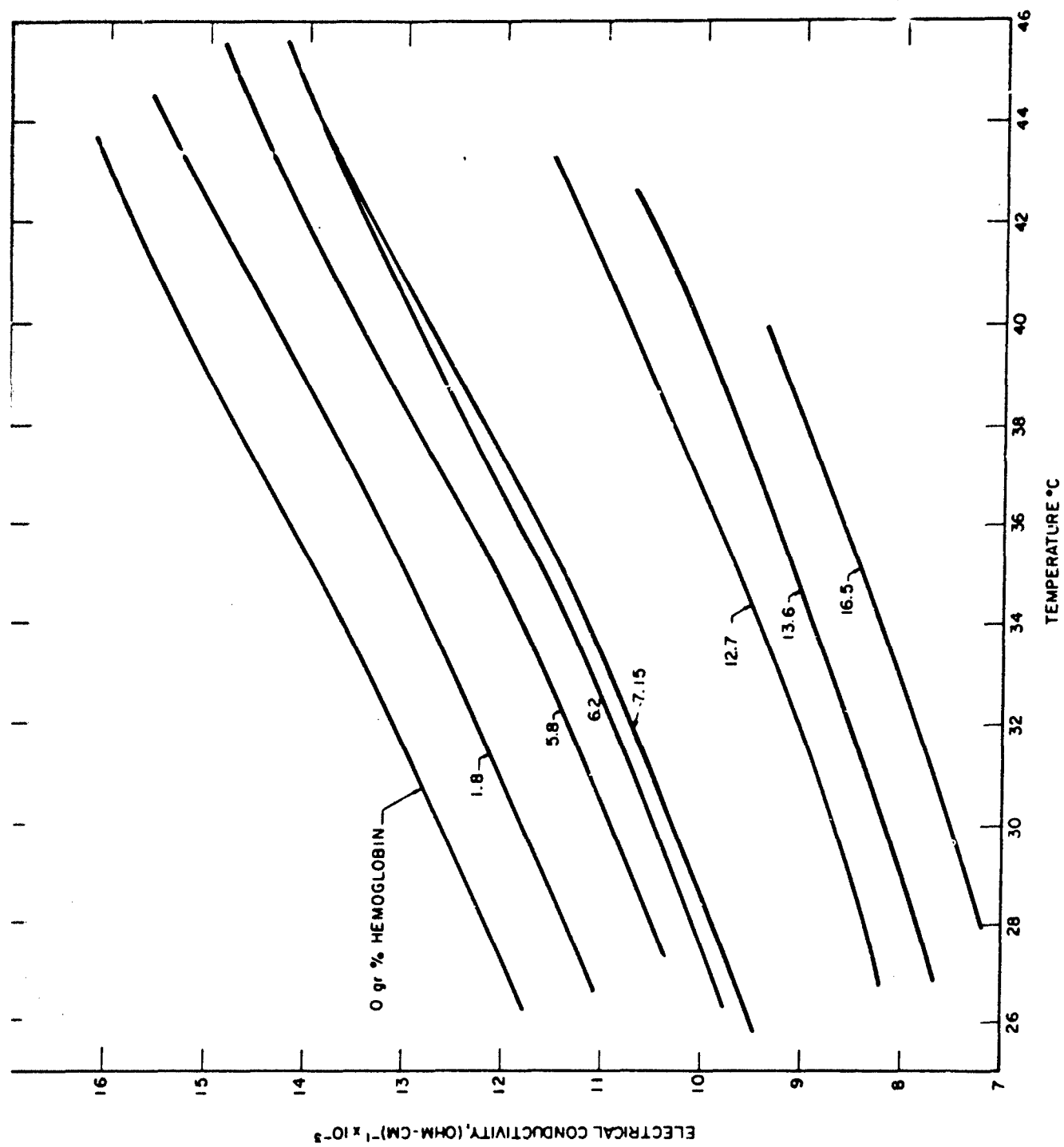


Figure 1. Electrical conductivity of bovine plasma with temperature.

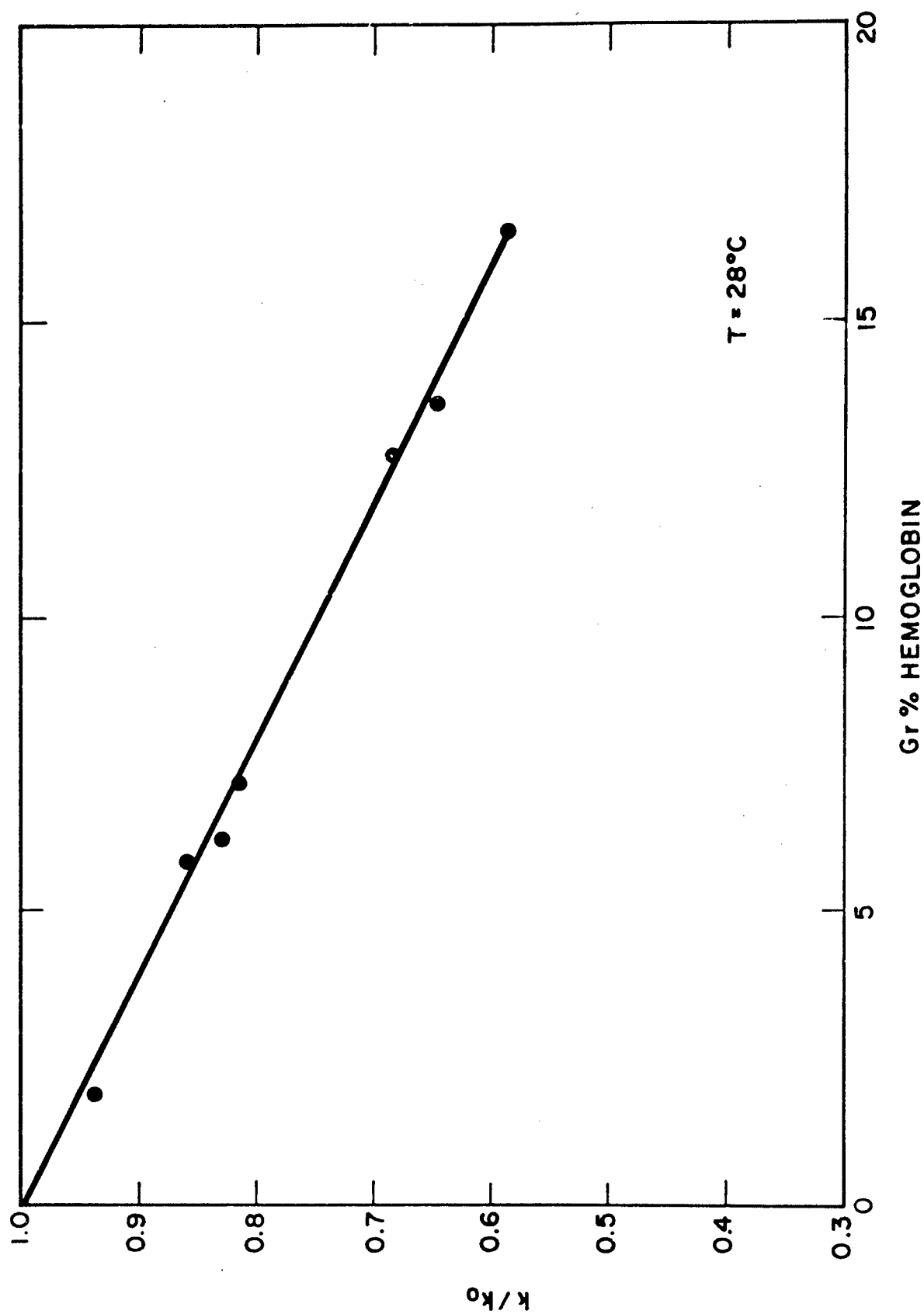


Figure 2. Dimensionless conductivity versus gram percentage hemoglobin content in bovine plasma.

color derivative. This substance is more dense than is cyanmethemoglobin, thus, the orthotolidine technique is much more sensitive than cyanmethemoglobin in the 0-1 gram/100 ml range.

Electrical resistance is measured with a Wheatstone bridge apparatus. The resistivity (ρ), is the resistance of a sample one cubic centimeter in size, and is related to resistance (R) by the equation:

$$R = \int_0^l \rho \frac{dl}{A} \quad (1)$$

where l is the length of the sample and A is the cross-sectional area. When the material has the same area along its entire length, the above formula reduces to

$$R = \rho \frac{l}{A} = \frac{l}{KA} \quad (2)$$

where conductivity is defined as the reciprocal of resistivity. In the case of a liquid conductor in a conductivity cell, the dimensions of the sample depend upon the geometry of the portion of the cell between the electrodes. For any particular conductivity cell, this is a measurable constant, and ρ can thus be calculated from Equation (2).

The first experiments were done with bovine blood. The electrical conductivity of each of the plasma hemoglobin dilutions was studied as a function of the temperature (See Figure 1). It is of interest to note, 1) the characteristic non-linear curve of an ionic solution between the ranges of 25°C and 46°C, and 2) the decrease in conductivity with increase in hemoglobin concentration.

Conductivity values at 28°C were plotted against their respective hemoglobin concentrations. The eight points fell in a well defined straight line. Similarly, for twelve samples of human blood, it was observed that for any individual sample, the points when plotted in this manner tend to fall in a straight line to about 12 gr Hb/100 ml solution. Beyond this point, the slope decreases. However, plasma conductivity varied between 1.6 and 12.9×10^3 (ohm-cm)⁻¹ among the persons tested (and from day to day in any particular individual). Thus, the position and the slope of the points was different for each

sample of blood measured. Some of these values are shown in Figure 3. Although the assumption that no hemoglobin was contained in the plasma is not entirely correct, a simple experiment showed that the amount of residual hemoglobin was less than the error involved in the cyanmethemoglobin technique.

In an attempt to find a more generalized, consistent, and useful relation than that presented in Figure 3, every conductivity value obtained by the above method was divided by its own pure plasma conductivity value (K_0) before being plotted. The K/K_0 values in Figure 4, therefore, pertain only to that sample. When plotted against hemoglobin concentration, the scatter in the ratio K/K_0 between individual samples is less than when the conductivities are plotted alone. The values fall within $\pm 5\%$ of a mean line, with the exception of only a few points.

An indication of the effect of the different plasma conductivities on the conductivity of the plasma/Hb solutions was obtained when the K_0 values were plotted versus the conductivity of a solution with 10 gr Hb/100 ml (again the K_{10} and the K_0 values are a set for each sample). Except for two points, a straight line was obtained. Thus, the plasma values from all the human series were averaged, and the resulting value was used as a common K_0 for all the remaining data. From a mean value, the variation amounted to approximately $\pm 2.5\%$.

These preliminary data seem to indicate that if other variables such as viscosity were included and the experiments were extended into the milligram range, a useful clinical blood damage criterion may evolve.

B. Other Biological Fluids

The electrical conductivities of human gastric juices, urine and bovine aqueous and vitreous humours were determined. These data are compared graphically in Figure 5 with representative values for human blood plasma. It may be noted that within the accuracy of the conductivity bridge ($\pm 0.10\%$), values for the aqueous and vitreous humours were identical. In view of the great difference in viscosities (a factor of 15-20) between the two latter fluids, it may be assumed that the vitreous humour possesses a higher compensating ionic concentration than the aqueous humour.

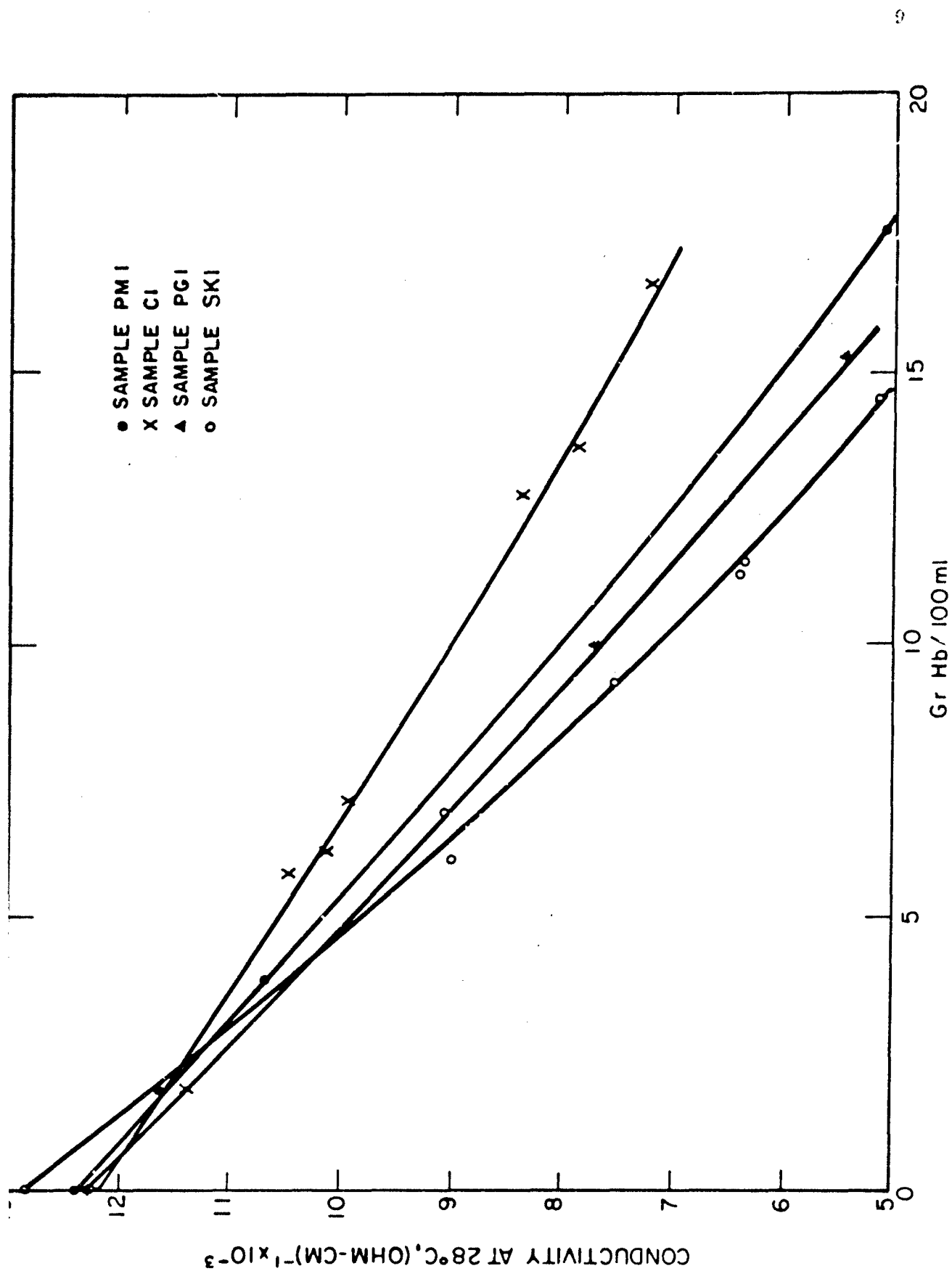


Figure 3. Electrical conductivity of human blood plasma versus Gr Hb/100 ml.

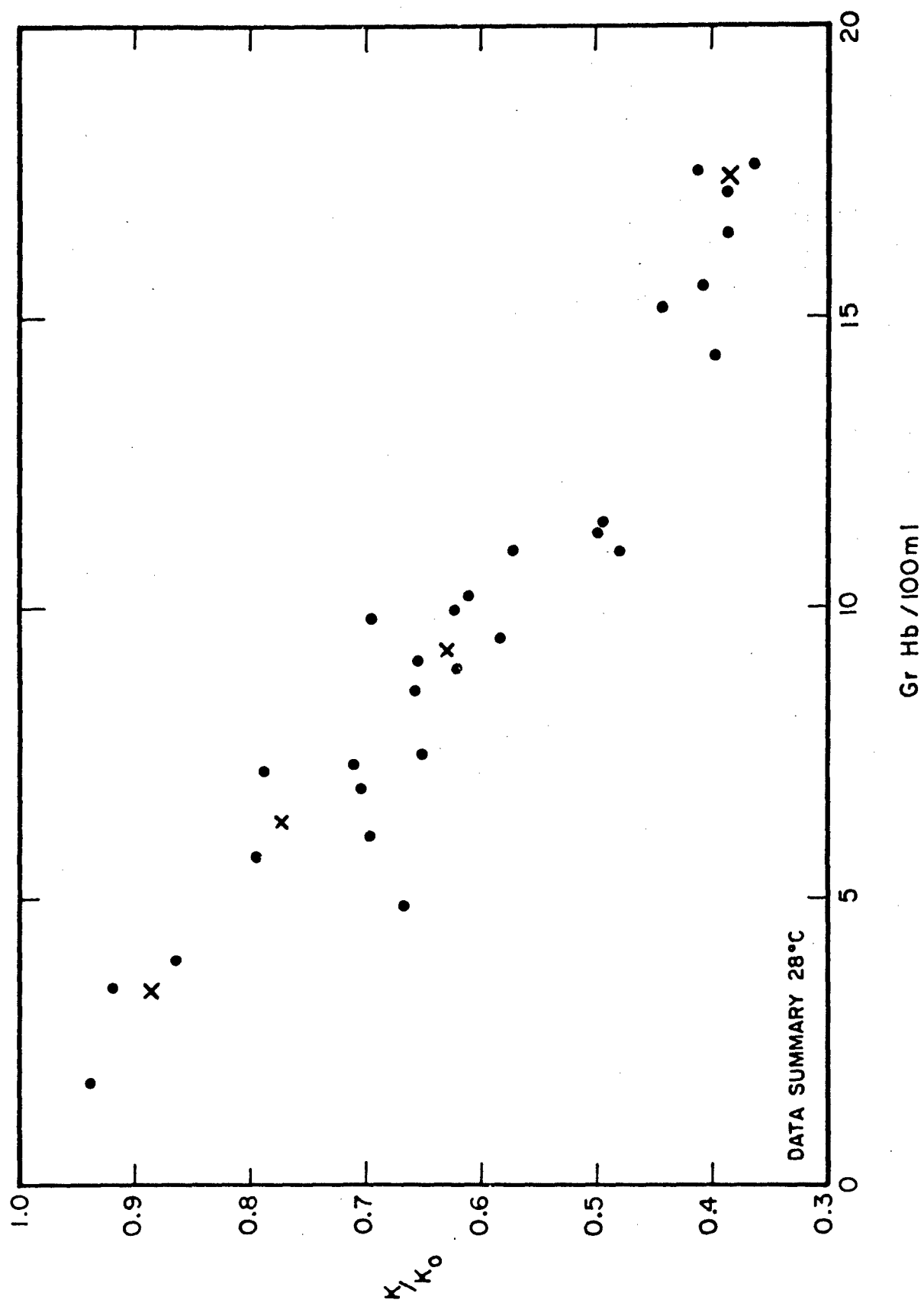


Figure 4. Normalized conductivity of human blood plasma with correction for individual variation.

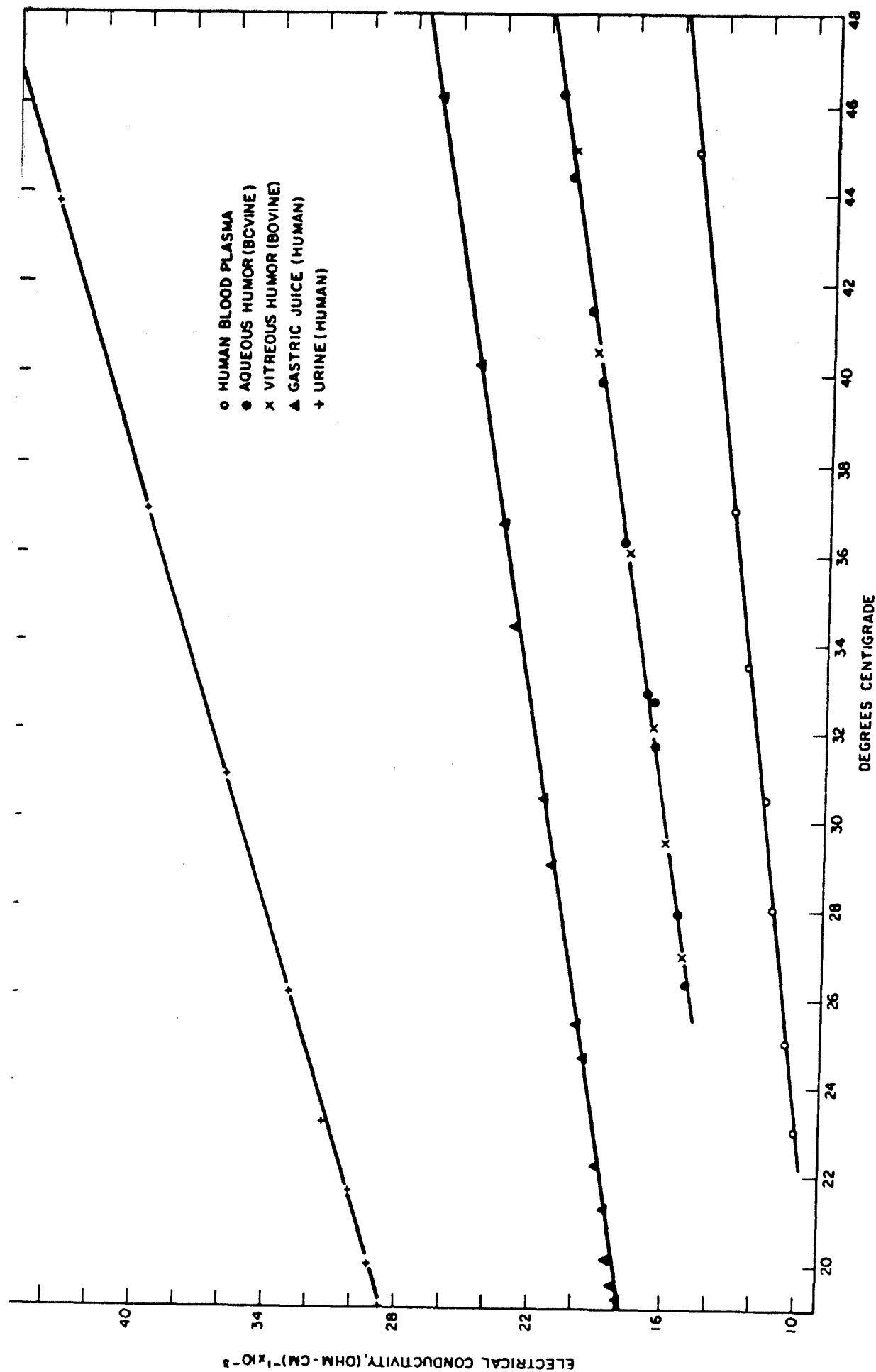


Figure 5. A summary of the electrical conductivities of several biological fluids.

The conductivity studies to date have shown urine to be the most highly conducting biological fluid.

The effects of temperature on the electrical conductivities of specific biological fluids have been measured. A summary of the fluids measured to date may also be seen in Figure 5, where the conductivity is presented as a function of temperature. As yet, no literature values for gastric juice and urine have been found, although the aqueous and vitreous humour values closely agree with what is given in Documenta Geigy⁽²⁾. Aqueous and vitreous humour do not seem to behave as strict Newtonian fluids because the difference in viscosity between them shows so little effect on the conductivity.

C. Biological Tissues

Two electrical conductivity cells designed expressly for the measurement of tissue conductivities were constructed (see Figures 6 and 7). The cell constants of the two instruments are 100 cm^{-1} and 60 cm^{-1} respectively. The larger device was designed to accept a range of tubular collimators having cell constants between 5 cm^{-1} and 200 cm^{-1} . The cells have been designed for use with the precision conductivity bridge or the standard potential-current (4 electrode) technique. Using both methods, data can be obtained to evaluate the extent of possible electrode interface resistance, or polarization errors. Both platinum electrode surfaces in contact with the biological tissues are platinized to reduce polarization errors.

Exploratory measurements have disclosed considerably lower conductivities for liver tissue than those for most biological fluids. Other measurements for tissue conductivities found in the literature verify the lower values obtained.

D. Thawed Whole Blood Preserved With PVP Additive

Frozen blood samples received from the Presbyterian Medical Center in San Francisco are frozen and thawed in accordance with the Linde process. Blood containing the PVP additive* is placed in a special Linde metal container which holds approximately 58 cc of blood. The blood is then quickly frozen by placing the metal container in a liquid

*Special additive composed of 60 ml ACDA, 437.5 gms of PVP K30 (polyvinylpyrrolidone) and sufficient 0.6 percent NaCl solution to make a total of 1000 cc.

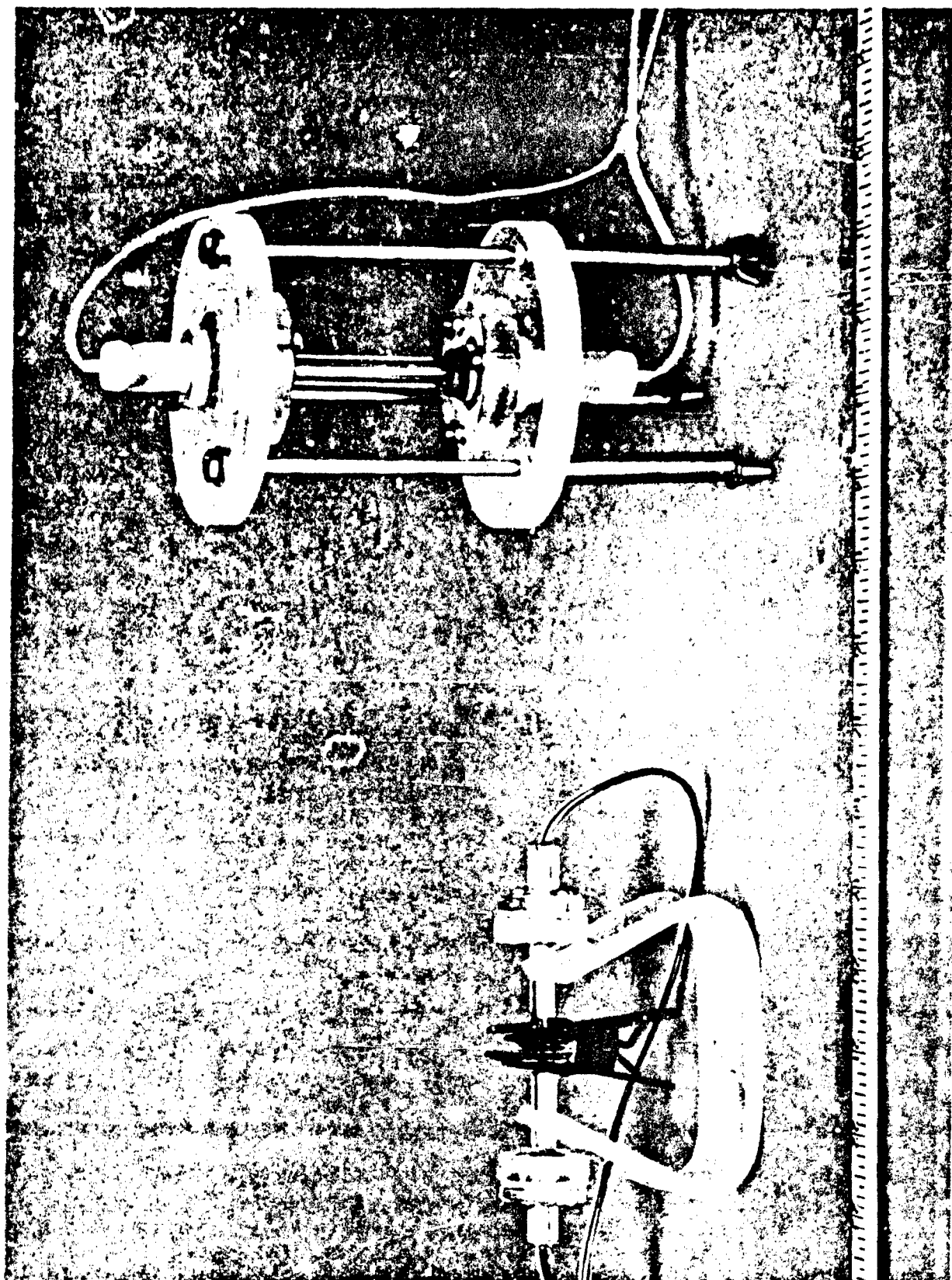


Figure 6. Tissue conductivity cells.

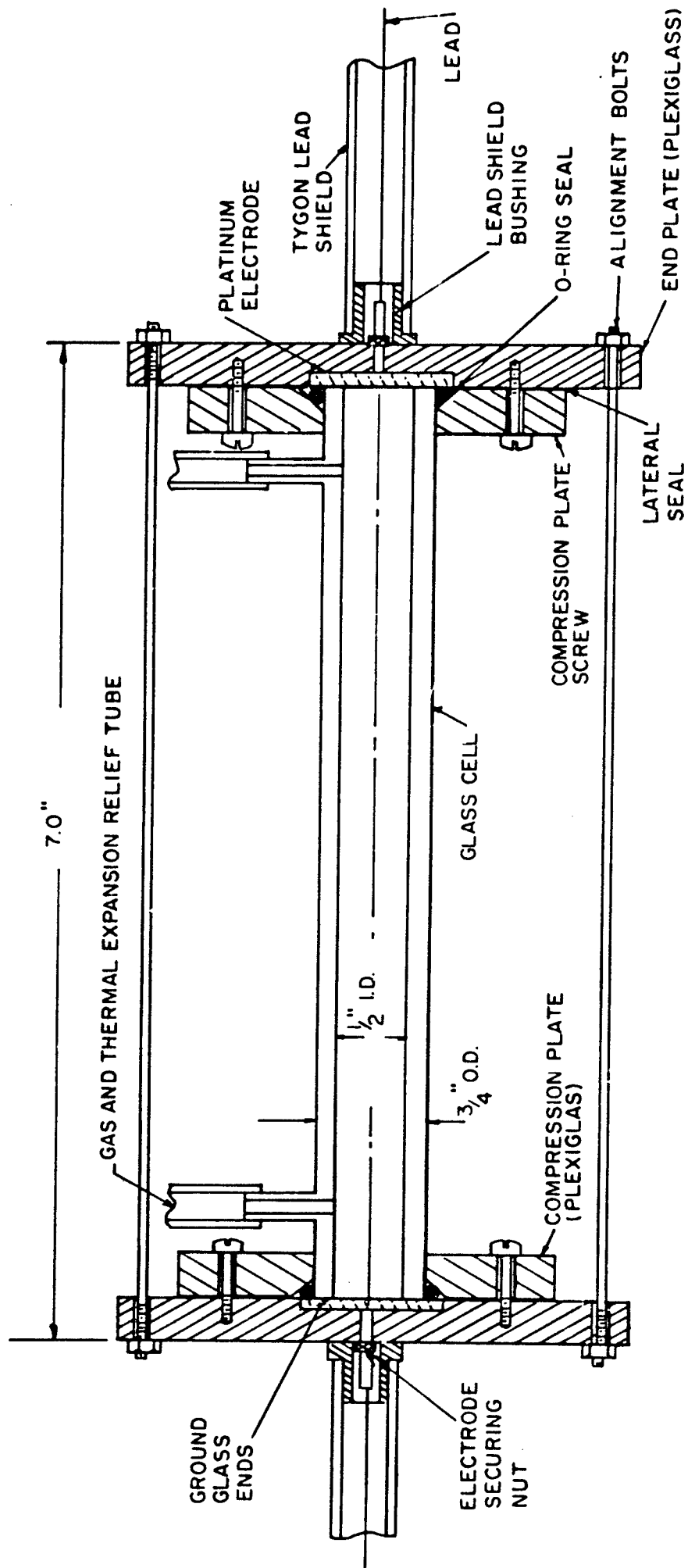


Figure 7. The biological tissues conductivity cell.

nitrogen bath. The frozen blood remains in the liquid nitrogen bath until it is to be used. Upon request, the metal container holding the frozen blood is placed in a special liquid nitrogen Dewar flask which is flown to Geoscience Ltd. The blood remains frozen in the Dewar flask for four to five days. Upon receiving the blood sample, the blood is thawed; the metal container is immersed rapidly in a water bath of 40°C, and the container is gently vibrated at a rate of 120 vibrations per minute. The blood is thawed in a 2-minute period.

The resulting electrical conductivity measurements of thawed blood containing PVP from the Presbyterian Medical Center are shown graphed in Figure 8 together with earlier measurements of unstressed whole blood without PVP additive. Note that the shape of the conductivity temperature curve between the two samples of blood are markedly different; also the magnitudes of the two values differ significantly at the higher temperature levels. It is not known at this time how much of these differences are attributable to the presence of the PVP additive. Studies to investigate this factor are currently in progress.

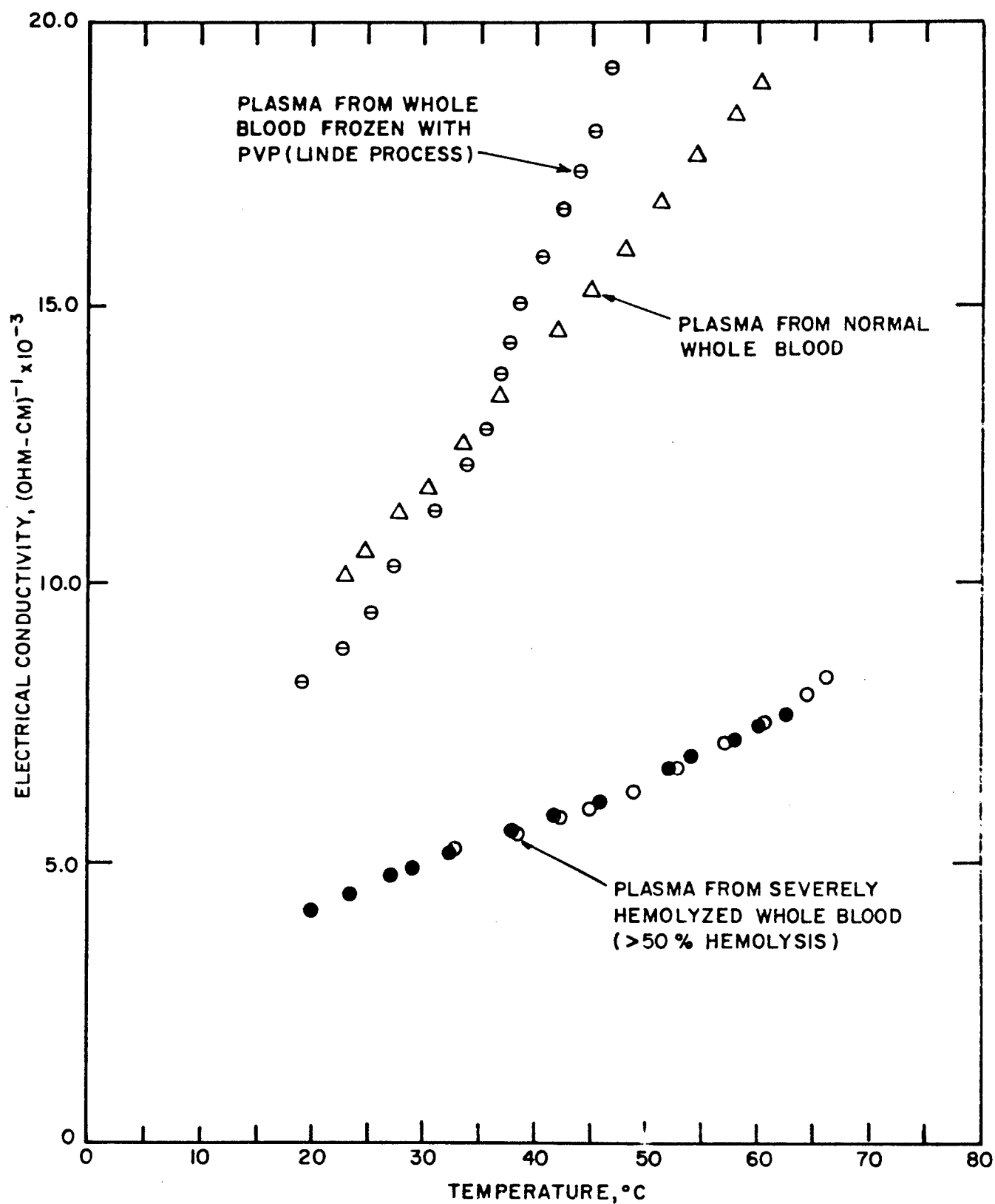


Figure 8. Comparisons of the electrical conductivities of blood plasma obtained from whole blood frozen with PVP and normal and hemolyzed blood plasma.

III. THERMAL CONDUCTIVITY

A. Biological Fluids and Tissues

The apparatus previously used for measuring the thermal conductivities of biological tissues and fluids⁽¹⁾ was modified somewhat before the current data were collected. Changes were made in the heating circuit; two flat electrical heating elements were used and a null heat meter inserted between them (see Figure 9). The electric powers for the two heaters were so adjusted that the heat flow through the null heat meter was zero; under these circumstances, the heat generated in the bottom heater was forced to flow through the cell containing the biological specimens. A piece of insulating material was placed between the top heater and the cooling plate to keep the current-voltage requirement of the top heater at a minimum. Spacers were added to the plastic insulation around the cell to insure correct positioning of the lower heating element. Because of the small difference in temperature between the cell and the container environment, the heat loss from the cell ends was small. The thermal conductivity cell itself consists of two highly conducting plates. The top plate of the cell was constructed with the heater attached to it, thus eliminating the error involved when the heater was not correctly positioned. Removable thermocouples were inserted in holes drilled through portions of the plates parallel to the cell surface. The top plate of the cell is removable so that the cell may be filled with biological specimens. These plates are spaced a short distance apart to maintain a large width to thickness ratio and thus insure one-dimensional heat flow through the cell.

The thermal conductivity apparatus was used to measure the conductivities of unstressed bovine lung, liver, kidney, brain, and aqueous and vitreous humour, and chicken skin. Measurements of the conductivity of distilled water were made periodically to check the accuracy of the system; all measurements for water were within $\pm 3\%$ of the established values.

Two other biological specimens were studied during this period. One sample was bovine brain (a repeat of some earlier work) and the other was bovine bone marrow. The thermal conductivity of bovine brain was found to be 0.24 Btu/hr ft²°F which is in general

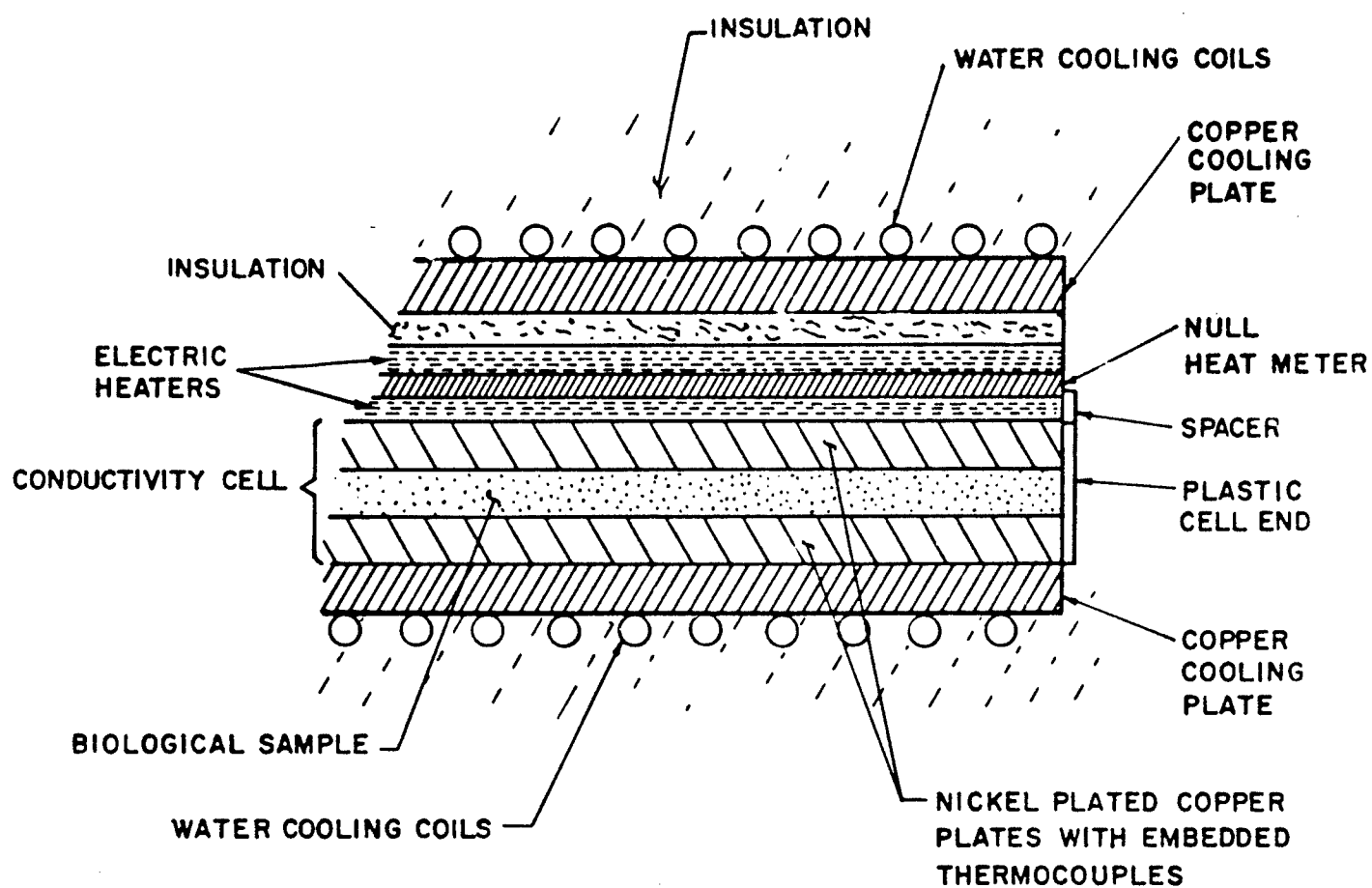


Figure 9. Cross sectional view of thermal conductivity cell.

agreement with values obtained earlier, specifically 0.28. The differences between these two values are thought to exist because visual differences in the color and texture of the two samples of brain were noted. The earlier measurements were made on specimens from a larger brain making it possible to obtain a more homogeneous sample almost totally white in color.

The thermal conductivity of bovine bone marrow was found to be 0.127. The marrow, a fatty substance, was removed from the bone and readily pressed into the thermal conductivity cell. Blood interspaced in the marrow could be seen to collect in tiny droplets on the surface as it was forced from the marrow. The surface color became pink. The density of the marrow was measured and found to be 0.92 grams per cc.

As a result of some of the thermal conductivity work, it is felt that small differences in thermal conductivity of a given biological specimen may occur as a result of small differences in chemical or physical structure. It is suggested that an additional parameter such as the density might be useful in further identifying the specimen. Consequently, an attempt will be made in all subsequent thermal and electrical conductivity measurements to also define the specimen in the terms of its density.

The thermal conductivities of biological specimens studied to date are shown in Table 1.

B. Thawed Whole Blood Preserved with PVP Additive

The determination of thermal conductivity of the thawed blood with PVP was found to be 0.231 Btu/hr ft² °F ft. It is noted that this value is significantly less than values obtained previously in this program for whole blood without PVP. This difference, however, may again be partially or mostly attributable to the PVP additive. At present the thermal conductivity of the PVP additive is in process of being measured since no values could be found in the literature. Preliminary results indicate that a large portion of the observed change is due to the additive. After the final information is available, a more detailed analysis of thermal conductivities of a mixture of blood and PVP will be made so that better interpretation of the low conductivity value for the mixture of blood and PVP can be assessed.

Table 1.

Thermal conductivity of
unstressed biological fluids and tissues
(75°F < t < 100°F)

Sample	Thermal Conductivity Btu/hr ft° F
Water	0.350
Beef Vitreous Humor	0.343
Beef Aqueous Humor	0.334
Plasma	0.330
Urine	0.324
Blood (hematocrit = 43%)	0.306
Beef Muscle	0.305
Beef Kidney	0.303
Beef Brain	0.287
Beef Liver	0.282
Gastric Juice	0.257
Chicken Skin	0.206
Beef Lung	0.163
Bone Marrow	0.127

C. Correlations

Attempts have been made to establish a correlation between the thermal conductivities of biological materials and another of their physical properties. Spells⁽³⁾ has proposed that a correlation exists between water content and thermal conductivity. For the materials which he included in his study, the correlation seemed to hold for samples with a water content of 50% or higher. When several additional biological materials were examined in the current study, however, and their thermal conductivities were plotted versus water content, the linear correlation was no longer found to hold. The most notable deviations occur for gastric juice, chicken skin and bovine lung samples with water contents greater than 70% (see Figure 10). Table 2 lists the values appearing in Figure 10 and the sources from which these values were obtained. Thus the water content correlation does not appear to be valid in a general sense. It is nevertheless a useful guide for estimating the thermal conductivity of biological materials. Further indication that the water content correlation is not an adequate one can be seen in an examination of the values obtained for aqueous and vitreous humours. The water content of these two samples varies by 0.01% and yet the thermal conductivity varies by 3%. Although these results are from preliminary data, the difference in thermal conductivity seems to be significant. This difference most probably is due to the difference in other constituents present in the two samples, and thus is in support of a correlation between the thermal conductivity of the whole sample and the thermal conductivity of the individual species present.

It would not be expected that a water content correlation would be general. Consider the case of lung tissue. In this specimen there exists a large volume of captured gas. Since the thermal conductivity of gases is considerably smaller than that for solids and liquids, this accumulation of gas must contribute to the low value of thermal conductivity obtained for lung tissue. It thus supports the idea that not only water content, but the content of all major species is important in correlating thermal conductivity values of various samples.

Bridgman⁽⁴⁾ derived a simplified expression which relates the thermal con-

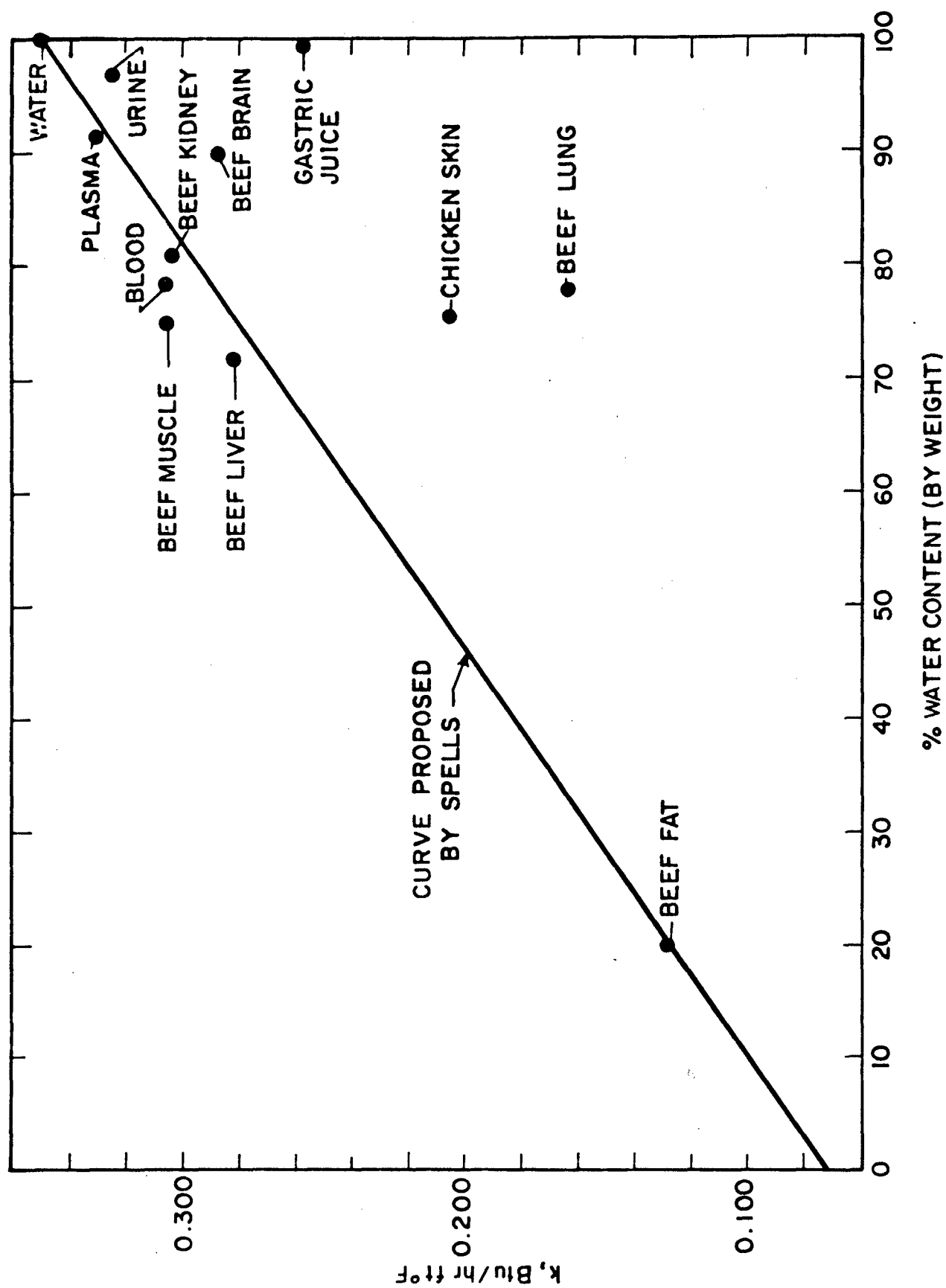


Figure 10. Thermal conductivity versus water content of biological tissues and fluids.

Table 2. Water Content and
Thermal Conductivity of Biological Samples

Sample	k _{experimental} *	% Water [†]	Source for % Water Content
Water	0.350	100.00	--
Human Plasma*	0.330	91.5(human)	Spells ³
Human Urine	0.324	97.0(human)	Langley & Cheraskin ⁵
Human Blood* (hematocrit = 43%)	0.306	78.5(human)	Spells
Ovine Muscle*	0.305	75.0(human)	Spells
Ovine Kidney*	0.303	81.0(rabbit)	Spells
Ovine Brain	0.287	90.0(human)	Documenta Geigy ²
Ovine Liver*	0.282	72.0(rabbit)	Spells
Human Gastric Juice	0.257	99.5(human)	Stuhlman ⁶
Chicken Skin	0.206	73.0(human)	Documenta Geigy
Ovine Lung	0.163	78.0(human)	Documenta Geigy
Ovine Fat	0.128	20.0(human)	Spells

All experimental values with the exception of bovine fat were measured at Geoscience:
the bovine fat value was determined by Spells.

It was not possible to obtain water contents for the exact specimens reported in the
thermal conductivity column. Values for similar types of specimens are presented in
this column.

ductivity to the velocity of sound and the molecular spacing. Therefore, it may be possible to describe the thermal conductivity of biological materials in terms of the bulk modulus or compressibility and the density.

Studies of thermal conductivity expressions which relate mean or overall thermal conductivity of a specimen to the conductivities of the individual constituents have been conducted. Specifically, three thermal conductivity models are being examined and tested. One model presumes that the species are positioned in laminae parallel to the heat flow, the second one presumes that the laminae are positioned normal to the heat flow and the third one presumes a uniform mixture of discrete groupings of the species (Eucken expression). It is recalled that the Eucken expression has been used previously in this program to determine the thermal conductivity of the erythrocytes from total whole blood and plasma conductivity measurements (knowing the hematocrit value). Further tests of the three models are in progress on simple mixtures. Specifically, the conductivities of water and alcohol mixtures as well as water and graphite mixtures have been predicted by the three methods and the results compared to available experimental information. One such comparison is shown in Figure 11 for ethyl alcohol and water mixtures. It is seen that the values determined from the Eucken expression are in good agreement with the experimental values. The model which postulates laminae which are parallel to the direction of heat flow yields values slightly above the experimental values, whereas, the model which postulates laminae which are perpendicular to the direction of heat flow (series model) yields values which are significantly below the experimental values. It is felt that the establishment of a realistic thermal conductivity model will be valuable not only for use in the correlation of the property measurements being made but also for the prediction of conductivities of specimens not yet studied.

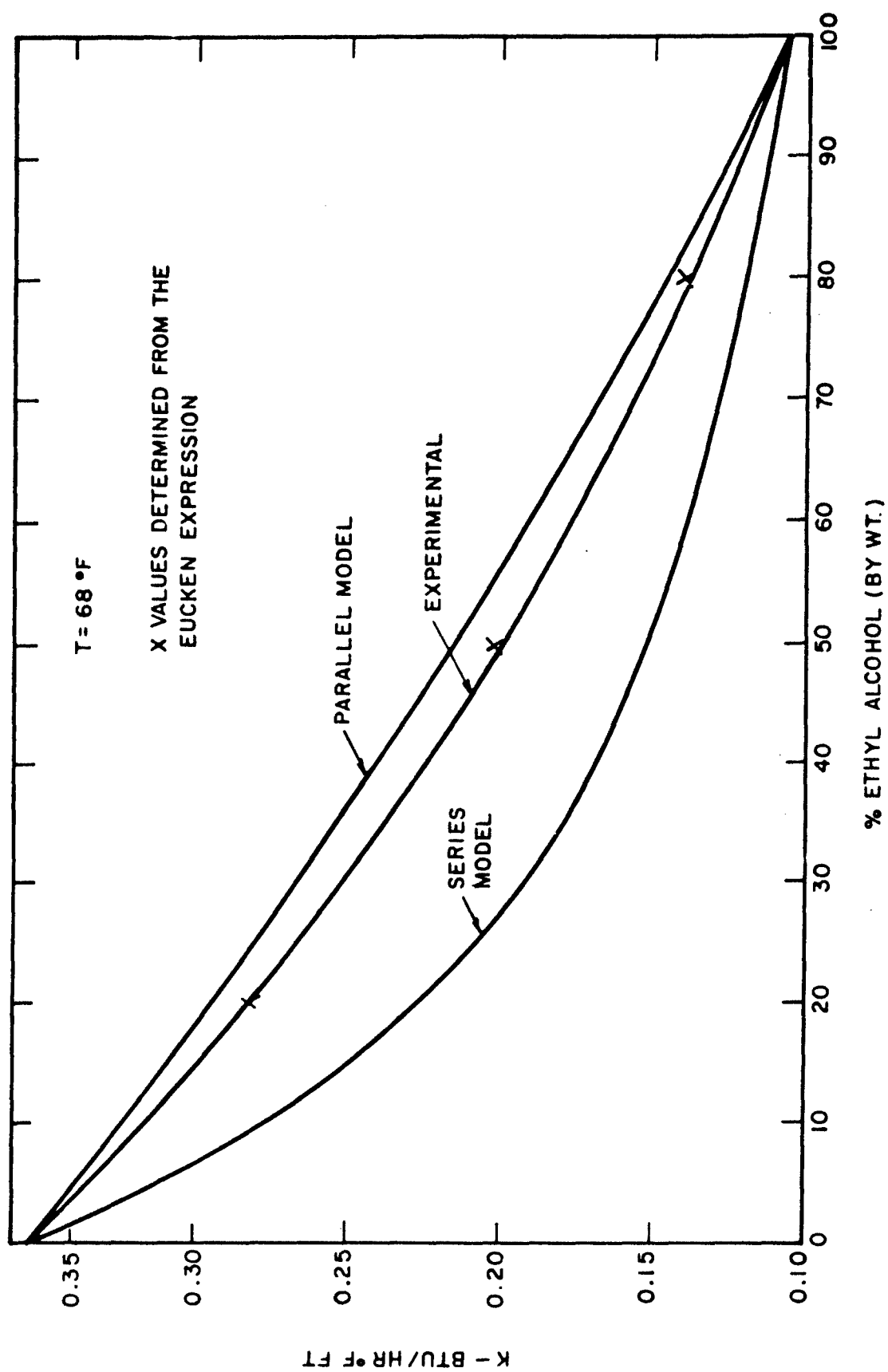


Figure 11. Thermal conductivity of ethyl alcohol mixed with water.

IV. CRYOGENIC EXPERIMENTS

Studies are now in progress to determine the effect of physical stresses on the thermal and electrical conductivity of biological samples. Freezing and thawing experiments are being conducted. Preliminary results for bovine liver show that a measurable change in thermal conductivity (from 0.282 to 0.305 Btu/hr ft° F) occurs when the sample has been frozen in liquid nitrogen and thawed quickly by immersion in warm water. When a sample was frozen slowly to 20° F, stored for two days, and then allowed to thaw slowly at room temperature a smaller change in conductivity was found than had been obtained for rapid freezing. However, these results are only based on one sample each and further data must be obtained before definite conclusions can be drawn.

A survey of the cryobiology literature has been conducted, (see attached bibliography) and three preliminary experiments were conducted as qualitative verification of some published information. Cowley, Timson, and Sawdye (see bibliography) reported that "the application of a thin layer of a thermally insulating material to containers were immersed in a cryogenic fluid such as liquid nitrogen". The effect of the coating was stated to be a function of the thermal resistance of the container material and its contents. To verify this, a small liquid container was constructed of two 4" square stainless steel plates, 1/16" thick, with the gap between them being 1/8". A Cu-constantan thermocouple, calibrated at liquid nitrogen temperatures, was connected to a transient recorder and immersed in the cell contents. Figure 12 shows the resulting curves for the uncoated cell and the cell when coated with vacuum grease: the results were found to agree with the findings of Cowley, et al.

In the second experiment, glass microhematocrit capillary tubes and 19 gauge hypodermic needles were filled with blood, sealed with crito-seal and immersed in liquid nitrogen until boiling had ceased. After thawing in 28°C water, the samples were centrifuged, and the degree of coloration of the plasma compared. No control of the cooling or warming rates was attempted, and in all cases the blood was severely damaged (up to 80% hemolysis).

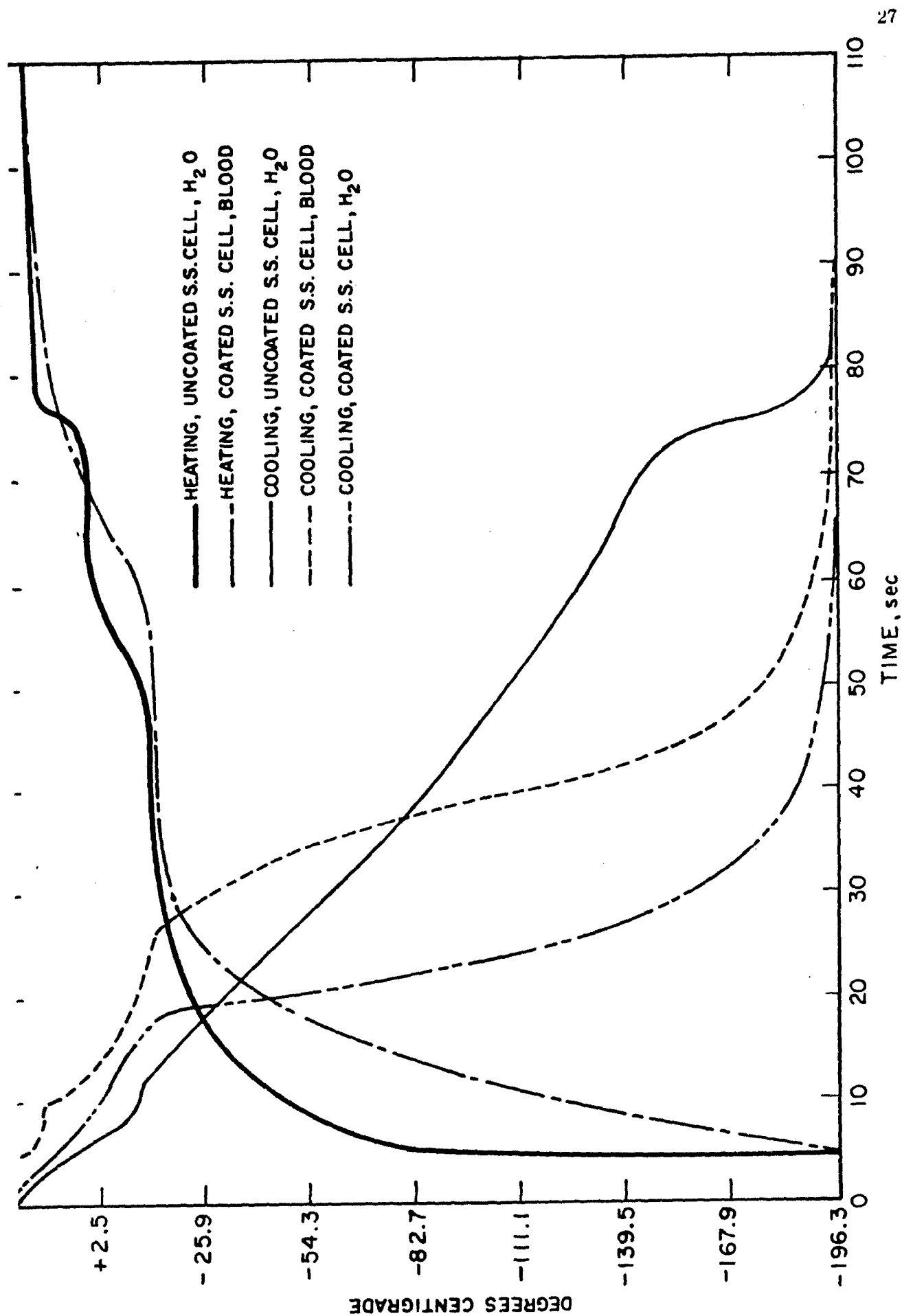


Figure 12. Cryogenic cooling and heating histories of blood and water.

Next, 3 " stainless steel capillaries were filled with blood, sealed with crito-seal, and subjected to various combinations of fast and slow freezing and thawing. Freezing and thawing rates were increased by agitating the tubes in the liquid nitrogen or 45°C water bath, since agitation increases the rate of heat transfer. Although all of the samples were again severely damaged, fast freezing and fast thawing proved to be the best combination for maximum survival of blood frozen without any preservative.

Excellent summaries of present progress of cryogenic studies and the theories and speculations involved can be found in Bibliographies 38 and 48.

V. STUDIES TO BE CONDUCTED DURING THE NEXT YEAR

The proposed research for the coming year may be divided into three areas: 1) measurement of the thermal and electrical conductivities of additional unstressed biological specimens, 2) continuing study of the aforesaid physical properties for biological specimens that have been exposed to cryogenic conditions, and 3) fundamental studies on rapid freezing and thawing techniques for biological specimens.

A. Thermal And Electrical Conductivity Measurements Of Unstressed Biological Specimens

In order to be able to interpret changes in physical properties of biological fluids and tissues that have been exposed to cryogenic conditions, it is necessary to obtain further data on unstressed specimens. The thermal and electrical conductivities determined will be compared and interpreted with the aid of analytical physical property models so that the results may be presented in the most general form.

B. Thermal And Electrical Conductivity Measurements of Biological Specimens Exposed to Cryogenic Temperatures

The electrical and thermal conductivities of specimens that have been stored at cryogenic temperatures for various periods of time will be determined. The changes in thermal and electrical properties determined for the stressed specimens will be presented in the form of indices which will be a measure of the morphological and biochemical changes. An attempt will be made to determine the effect of storage time in addition to freezing and thawing times.

C. Fundamental Studies On Rapid Freezing And Thawing Techniques

Literature searches and discussions with leading authorities in the area of biological material damage during cryogenic freezing and ensuing thawing indicate an important need for a more comprehensive and quantitative understanding of these processes. A number of freezing and thawing experiments are proposed which it is felt will define the more important, controlling parameters.

1. Freezing Methods. It is thought that one biological damage mechanism in

cryogenic freezing is the freezing rate (the low rates creating more cell damage). Therefore, ways in which the freezing rate can be enhanced or increased will be studied. An investigation will be made of blood cell recovery as a function of total freezing times. Another experiment would consist of freezing a thick cylinder of blood by forced convection cryogenic boiling such that no film boiling exists. The freezing rate would decrease toward the center of the cylinder. Cell recovery rates would be measured after thawing in the same manner; the results should give quantitative information on the influence of freezing and thawing rates on hemolysis.

2. Thawing Methods. In the opinion of workers in the field of blood preservation, frozen blood cell survival is also dependent upon thawing rates. Qualitatively it was observed that the shorter the time required for thawing, the greater the cell survival rate. It is proposed that thawing be produced by a resistance heating method in which high frequency electrical currents are conducted through a platinum cell containing frozen blood pellets or flakes. When the blood becomes molten in any localized region of the cell, the conductivity increases by a few orders of magnitude in that region. Thus, the voltage differential across the region decreases, and the volumetric power generation decreases. A greater voltage then appears across adjacent unthawed portions, thereby, selectively heating in a uniform fashion. Total times required for thawing blood samples would be studied as a function of percentage cell survivals.

In addition to the above program, two papers are being prepared for publication in the open literature; one reports thermal conductivity research for unstressed biological materials and the other summarizes the corresponding work on electrical conductivity.

VI. REFERENCES

1. Poppendiek, H.F., Greene, N.D., Breeden, J.H., Murphy, J.R., Morehouse, P.M., Feigenbutz, L.V., Hody, G.L., and Hayes, J.R., Annual Report on "Thermal and Electrical Conductivities of Biological Fluids and Tissues," GLR-24, April 1, 1963 to March 31, 1964.
2. Documenta Geigy, 5th Edition, N.Y. 1959
3. Spells, K.E., "The Thermal Conductivities of Some Biological Fluids," Physics in Medicine and Biology, Nov. 1960.
4. Bridgeman, P.W., Proc. Nat. Acad. of Sciences 9,341; 1923.
5. Langley, L.L. and Cheraskin, E., The Physiology of Man, 2nd Edition, N. Y. 1958.
6. Stuhlman, Otto, Jr., An Introduction to Biophysics, N.Y. 1943.

VII. CRYOGENIC BIBLIOGRAPHY

1. Albright, J. F., et al., "Preservation of Antibody Producing Cells at Low Temperature: A Method of Storage that Allows Complete Recovery of Activity." Proc. Soc. Exp. Biol. Med. 114: 489-93, Nov., 1963
2. Blackshaw, A. W., "Preservation of Rabbit, Sheep, and Ox Red Cells at -79°C ." Aust. J. Biol. Sci., 7: 566-572, 1954
3. Cowley, C. W., W. J. Timson, and J. A. Sawdye, "Ultra Rapid Cooling Techniques in the Freezing of Biological Materials," Biodynamica 8: #170, Dec., 1961
4. Dobry, E., and L. Livora, "Preservation of Erythrocytes at below Freezing Temperatures in Dextran Medium" Cas Lek Cesk 101: 1074-5, 31 Aug. 1962
5. Evans, V. J., et al., "Recovery from Liquid Nitrogen Temperature of Established Cell Lines Frozen in Chemically defined Medium," J. Nat. Cancer Inst. 29: 749-57, Oct., 1962
6. Farr, A. D., "A Comparative Study of the Insulating Properties of Four Common Blood Transport Containers," Vox Sang 7: 504-12, Jul-Aug., 1962
7. Feder, N., and R. L. Sidman, J. Biophys. Biochem. Cyt. 4: 593, 1958
8. Gehenio and Luyet, Proc. Roy. Soc. London 147: No. 929, 1957
9. Gehenio, P. M., and B. J. Luyet, "Prevention of Hemolysis in Frozen Blood by Rapid Freezing and Thawing," Bibliotheca Haematologica (Basel) Fasc 7: 330, 1958

0. Gehenio, P. M., and B.J. Luyet, Fed. Proc., 17: 52, 1958
1. Gehenio, P.M., et al., "Effects of Freezing Velocities in Causing or Preventing Hemolysis," Biodynamica 9: 77-82, Oct., 1963
2. Greaves, R. I., et al., "Preservation of Living Cells by Freezing and by Drying," Fed. Proc., 22: 90-3, Jan-Feb 1963
3. Green, I.J., "The Preservation of Selected Mammalian Cells by Freezing and Storage at -64°C. I. Studies on the Short-Term Preservation of Eight Mammalian Cell Strains," J. Formosa Med. Ass. 61: 527-36, 28 Jan., 1962
4. Haynes, L. L., et al., "Long-Term Blood Preservation - A Reality," J. Mich Med. Soc. 61: 1509-12, Dec., 1962
5. Huggins, C. E., "Reversible Agglomeration used to Remove Dimethylsulfoxide from large Volumens of Frozen Blood," Science 139: 504-5, 8 Feb., 1963
6. Huggins, C. E., "Preservation of Blood for Transfusion by Freezing with Dimethylsulfoxide and a Novel Washing Technique," Surgery 54: 191-4 Jul., 1963
7. Huntsman, R. G., et al., "Blood Groups and Enzymes of Human Red Cells after a Year's Storage in Liquid Nitrogen," Brit. Med. J., 5318: 1508-14, 8 Dec., 1962
8. Huntsman, R. G., et al., "Liquid Nitrogen Storage of Hemoglobin Variants," J. Clin. Path. 17: 99-100, Jan., 1964
9. Hurn, B. A., H. Lehmann, and R. G. Huntsman, "Thawing of Blood Stored in Liquid Nitrogen by Means of Radio-Frequency Heating," Transfusion, 2: 265, Jul-Aug, 1962

20. Iossifides, I., et al., "Preservation of Clot Retracting Activity of the Platelets by Freezing in Dimethylsulfoxide and Plasma, " Transfusion, 3:167-72, May-Jun.,, 1963
21. Jacobs, M.H., "Surface Properties of the Erythrocyte, " Ann. N.Y.Acad. Sci. 50: 824-833, 1950
22. Jacobs, M.H., H.N. Glassmann, and A.K. Parport, "Glycerol (3%) as a Hemolytic Agent, " J. Exp. Zool., 113: 277, 1950
23. Lovelock, J.E., "The Mechanism of the Protective Action of Glycerol against Haemolysis by Freezing and Thawing, " Biochem. Biophys. Acta 11: 28-36, 1953
24. Lovelock, J.E., "Biophysical aspects of Freezing and Thawing of Living Cells, " Proc. Roy. Soc. Med. 47: 60-2, 1954
25. Lovelock, J.E., "Haemolysis by Thermal Shock, " Brit. J. Haematol. 1: 117-29, 1955
26. Lozina-Lozinskii, L.K., [Resistance of some Insects to the Temperature of Liquid Helium (-269°) in Conditions of Intracellular Freezing in the Absence of Antifreezes] Tsitologiya 5: 220-30, Mar-Apr., 1963 (Russ)
27. Luena, C.V., and D. Rose, Arch. Bioch. Bioph., 65: 534, 1956
28. Luyet, B.J. and P.M. Gehenio, "Some Aspects of the Problem of the Relationship between Hemolysis and Freezing Velocity, " Anat. Record 125: 615, 1956
29. Luyet, B.J., Biodynamica 6: 217, 1949
30. Luyet, B.J. and P.M. Gehenio, Biodynamica 6: 273, 1955

31. Luyet, B.J., and L.J. Menz, Biodynamica 7: 25, 1951
32. Luyet, B.J., "On the Mode of Action of Rapid Cooling in the Preservation of Erythrocytes in Frozen Blood, " Biodynamica 9: 95-124, Oct., 1963
33. Mackenzie, A.P., et al., "An Electron Microscope Study of the Fine Structure of Very Rapidly Frozen Blood Plasma, " Biodynamica 9: 147-64, Oct., 1963
34. Meryman, H.T., "Freezing and Drying of Biological Tissues, " New York Academy Science Annals 85: Art 2, 1960
35. Meryman, H.T., "Preservation of Living Cells, " Fed. Proc. 22: 81-9, Jan-Feb, 1963
36. Meryman, H.T., and E. Kafig, Proc. Soc. Exp. Biol. and Med. 90: 587, 1955
37. Meryman, H.T., "Tissue Freezing and Local Cold Injury, " Physiological Reviews 37: 233-51, 1957
38. Meryman, H.T., "Mechanics of Freezing in Living Cells, and Tissues, " Science 124: 515, 1956
39. Nath, S., et al., "An Electron Microscope Study of the Distribution of Ice in Frozen Blood Plasma " Biodynamica 9:137-46, Oct., 1963
40. Pegg, D.E., "In Vitro Assessment of Cell Viability in Human Bone Marrow Preserved at -79°C, " J. Appl. Physiol. 19: 123-6, Jan, 1964
41. Raby, C., [Changes in Blood Collected on Heparin According to the Preservation Temperature], Hemostase 2: 295-303, Dec., 1962 (Fr.)

42. Rapatz, G., et al., "Electron Microscope Study of Erythrocytes in Rapidly Frozen Mammalian Blood," Biodynamica 9: 83-94, Oct., 1963
43. Rapatz, G., et al., "Effects of Cooling Rates on the Preservation of Erythrocytes in Frozen Glycerolated Blood," Biodynamica 9: 125-36, Oct., 1963
44. Rimon, A., et al., "Biochemical and Electron Microscope Study of Preserved Erythrocytes," Transfusion 3: 161-6, May-Jun, 1963
45. Rinfret, A.P., "Factors Affecting the Erythrocyte During Rapid Freezing and Thawing," Ann. N.Y. Acad. Sci. 85: 576-594, 1960
46. Rinfret, A.P., "Some Aspects of Preservation of Blood by Rapid Freeze-Thaw Procedures," Fed. Proc. 22: 94-101, Jan-Feb., 1963
47. Sherman, J.K., "Questionable Protection by Intracellular Glycerol During Freezing and Thawing," J. Cell. Comp., 61: 67-83 Feb, 1963
48. Smith, A.U., Biological Effects of Freezing and Supercooling, Williams & Wilkins, Baltimore, 1960
49. Smith, A.U., "Prevention of Hemolysis During Freezing and Thawing of Red Blood Cells," Lancet 259: 910, 1950
50. Strumia, M.M., Science 110: 398, 1949
51. Strumia, M.M., L.C. Colwell, and P.V. Strumia, "Preservation of Whole Blood in Frozen State for Transfusion," Science 128: 1002, 1958

- . Taylor, A.C. and R. Gerstner, "Tissue Survival After Exposure to Low Temperatures and the Effectiveness of Protective Pretreatment," J. Cell. Comp. Physiol., 46: 477-502, 1955

- . Tomity, I., et al., [The Effect of Freezing on the Submicroscopic Structure of the Myelin Sheath], Kiserl Orvostud 15: 539-45, Oct., 1963 (Hun)